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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :		(11) International Publication Number:	WO 96/12022
C12N 15/24, C07K 14/54, C12N 5/10, A61K 38/20	A1	(43) International Publication Date:	25 April 1996 (25.04.96)

(21) International Application Number: PCT/EP95/04023

(22) International Filing Date: 12 October 1995 (12.10.95)

(30) Priority Data:
MI94A002097 13 October 1994 (13.10.94) IT

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(81) Designated States: AU, BR, BY, CA, CN, EE, FI, JP, KR, KZ, LT, LV, MX, NO, RU, SG, SI, UA, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: INTRACELLULAR ISOFORM OF THE INTERLEUKIN-1 RECEPTOR ANTAGONIST

(57) Abstract

It is described a new interleukin-1 antagonist active both against IL-1a and IL-1B, a new DNA sequence encoding the IL-1 antagonist and the method for obtaining a IL-1 antagonist by the recombinant DNA technique; it is also described the prophylactic, therapeutic and diagnostic use of such new IL-1 antagonist in pathologies deriving from the IL-1 production.

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Intracellular isoform of the interleukin-1 receptor antagonist

FIELD OF THE INVENTION

The present invention is in the field of biotechnology. It is described a new interleukin-1 (IL-1) antagonist active both against IL-1a and IL-1B, a new DNA sequence encoding the IL-1 antagonist and the method for obtaining a IL-1 antagonist by the recombinant DNA technique. It is also described the prophylactic, therapeutic and diagnostic use of such new IL-1 antagonist in pathologies deriving from the IL-1 production.

BACKGROUND OF THE INVENTION

There are two distinct genes encoding the interleukin-1 (IL-1) named IL-1a and IL-1B, which encode protein IL-1a and IL-1B respectively.

Interleukin IL-1a and IL-1B are pleiotropic cytokines, which, although their sequences show scarce analogy, exert a variety of similar effects on different tissues and act on many human pathologies, in particular on the immunitary response of the organism and on inflammatory processes.

Both the proteins have a molecular weight of about 17.5 KDa and have been previously synthesised as precursor molecule of larger size having a molecular weight of about 31 KDa.

IL-1s are potent inflammatory and pyrogenic cytokines that normally have beneficial effects but can also have extremely unhealthy effects for the organism.

They can, for example, participate in the pathogenesis of symptoms of the autoimmune pathologies like lupus eritematosus and, in particular, they are involved as mediators to provoke damages to tissues as for example in rheumatoid arthritis.

Many of the biological effects of IL-1 are similar to those that can be observed during a septic event. Recent studies demonstrated that the endovenous administration of IL-1 in doses from 1 to 10 ng/kg gives rise to fever, sleepiness, anorexia, generalised myalgia, arthralgia and cephalea.

Since IL-1 have pleiotropic biological activities, many of which influence negatively the organism, the powerful effects of IL-1 should be under strict physiological control.

IL-1 synthesis is inhibited by anti-inflammatory cytokines, prostaglandins and glucocorticoids and the existence of multiple levels of inhibition of IL-1 points to the necessity of a strict control of this mediator.

IL-1 is the only cytokine for which an antagonist polypeptide for the receptor has been described up to now: the third known component until today of the IL-1 family is the antagonist for the IL-1 receptor (IL-1ra).

All three components (IL-1a, IL-1B, IL-1ra) recognise and bind to the same receptor on cell surface (IL-1R); IL-1a and IL-1B binding to IL-1R transmit a signal, whilst IL-ra does not.

There are two types of IL-1 receptors named IL-1RI and IL-1RII. IL-1ra is a polypeptide which binds IL-1RI, and with less affinity IL-1RII, without any agonistic activity.

IL-1ra production is induced in different cellular types, including mononuclear phagocytes, polymorphonuclear cells (PMN) and fibroblasts, by IgG, cytokines and bacterial products.

Until now two molecular forms of IL-1ra have been identified and cloned: 1) secreted IL-1ra (sIL-1ra) contains a classical leader sequence of 25 amino acids giving a mature protein of 152 amino acids; 2) intracellular IL-1ra (icIL-1ra) lacks a leader sequence thus predicting that this protein remains intracellular.

sIL-1ra and icIL-1ra are generated from the same gene. icIL-1ra transcripts originate from an alternative starting site and from the splicing of a first alternative exon into an internal splice acceptor site located in the first exon of sIL-1ra. The predicted proteins are thus identical except in their NH_2 ends, where the first 21 amino acids of sIL-1ra are substituted by four amino acids in icIL-1ra.

Expression of transcripts encoding sIL-1ra and icIL-1ra is differently regulated. The biological significance of icIL-1ra is still unclear.

Considering that IL-1 is involved in pathogenesis of many diseases it is evident the need of having available medicaments useful to limit the unhealthy effects of IL-1.

SUMMARY OF THE INVENTION

An object of the present invention is to provide an IL-1 antagonist active against both IL-1a and IL-1B and against a combination of them.

A further object of the present invention is to provide a DNA sequence encoding an IL-1 antagonist and a method for obtaining such new antagonist by the recombinant DNA technique.

Another further object of the present invention is to provide the antagonist in substantially purified form in order to be suitable for use in pharmaceutical compositions active in pathologies that require IL-1 inhibition.

Further objects and advantages of the invention will be evident in the following description.

BRIEF DESCRIPTION OF THE FIGURES AND SEQUENCE LISTING

Figure 1 describes the DNA sequence and the sequence of the protein, for the portion not in common, of icIL-1raII (SEQ ID NO:8 and SEQ ID NO:9) compared to those of classic sIL-1ra (icIL-1raI; SEQ ID NO:6) and of sIL-1ra (SEQ ID NO:4 and SEQ ID NO:5), and it further describes the DNA sequence and the encoded protein for the portion of IL-1ra in common (SEQ ID NO:13 and SEQ ID NO:14).

Figure 2 describes the RT-PCR analysis of icIL-1raII expression in different cell types.

Figure 3 describes the Western blot analysis of recombinant icIL-1raII.

Figure 4 describes the effects of icIL-1raII on IL-1 induced expression of E-selectin in endothelial cells.

SEQ ID NO:1 reports the sequence of an oligonucleotide named IRA5 for use in RT-PCR.

SEQ ID NO:2 reports the sequence of an oligonucleotide, corresponding to nucleotides 69-70 of B-actin cDNA, for use in RT-PCR.

SEQ ID NO:3 reports the sequence of a backward oligonucleotide, complementary to nucleotides 430-449, for use in RT-PCR.

SEQ ID NO:4 reports the DNA sequence encoding sIL-1ra for the portion not in common.

SEQ ID NO:5 reports the amino acid sequence of sIL-1ra for the portion not in common.

SEQ ID NO:6 reports the DNA sequence encoding three amino acids of icIL-1raI for the portion not in common.

SEQ ID NO:7 reports the three amino acids of icIL1-raI for the portion not in common.

SEQ ID NO:8 reports the DNA sequence encoding icIL-1raII for the portion not in common.

SEQ ID NO:9 reports the amino acid sequence of icIL-1raII for the portion not in common.

SEQ ID NO:10 reports the DNA sequence encoding IL-1ra for the portion in common. With regard to questions related to the "Patentin EPO" program, for the preparation of the sequences a G nucleotide was added in the first position of the sequence in order to permit the encode of the first amino acid Glu and further in order to avoid the formation of a stop codon in the inner side of the sequence.

SEQ ID NO:11 reports the amino acid sequence of IL-1ra for the portion in common.

SEQ ID NO:12 reports the sequence of 21 amino acids representing a icIL-1raII fragment not in common with the other IL-1ras.

SEQ ID NO:13 reports the DNA sequence encoding the complete icIL-1raII.

SEQ ID NO:14 reports the amino acid sequence of complete icIL-1raII.

DESCRIPTION OF THE INVENTION

This new IL-1 antagonist was generated by inserting in the frame of the DNA encoding icIL-1ra a new 63 base pairs (bp) sequence between the first icIL-1ra specific exon and the internal acceptor site of the first exon of sIL-1ra.

By RT-PCR experiments the present inventors found that this new transcript is expressed in activated monocytes and fibroblasts and in polymorphonuclear cells (PMN).

Expression in COS cells revealed that this new antagonist is mostly intracellular and has a molecular weight (MW) of approximately 25 KDa in SDS-PAGE.

The new recombinant antagonist shows IL-1 inhibitory activity.

In the present application, for reason of clearness and easiness, the presently known icIL-1ra are indicated as icIL-1ra type I (icIL-1raI), whereas the new antagonist here described and object of the present invention is defined as icIL-1ra type II (icIL-1raII).

Examples of pathologies in which the new antagonist according to the invention can be advantageously used for prophylactic, therapeutic or diagnostic use are rheumatoid arthritis, septic shock, acute myelomonocytic leukaemia, immunological reaction of transplantation against host, acquired immunodeficiency syndrome (AIDS), ulcerative colitis and all autoimmune diseases in general.

An embodiment of the invention is the administration of a pharmacological active amount of icIL-1raII to people having a high risk to

develop pathologies requiring IL-1 inhibition or to people already showing pathologies like sepsis.

An example of the category above cited are patients waiting for a surgical operation.

Any route of administration compatible with the active principle can be used, but particularly preferred is the parenteral administration because it permits to have, in short times, systemic effects.

For this reason, it is preferable the administration of a endovenous bolus just before, during or after the surgical operation. The dose of icIL-1raII to be administered depends on the basis of the medical prescriptions according to age, weight and the individual response of the patient.

The dosage can be between 0.05 and 30 mg/Kg body weight and the preferable dose is between 0.1 and 10 mg/Kg body weight.

The pharmaceutical composition for parenteral use can be prepared in injectable form comprising the active principle and a suitable vehicle. Vehicles for the parenteral administration are well known in the art and comprise, for example, water, saline solution, Ringer solution and dextrose.

The vehicle can contain smaller amounts of excipients in order to maintain the solution stability and isotonicity.

The preparation of the cited solutions can be carried out according to the ordinary modalities and preferably the icIL-1raII content will be comprised between 1 mg/ml and 10 mg/ml.

Further examples of pathologies wherein the new antagonist according to the invention can be advantageously used for prophylactic, therapeutic diagnostic purpose are rheumatoid arthritis, septic shock, acute myelomonocytic leukaemia, immunological reaction of transplantation against host, acquired immunodeficiency syndrome (AIDS), ulcerative colitis and all autoimmune diseases in general.

The present invention has been described with reference to the specific embodiments, but the content of the description comprises all modifications and substitutions which can be brought by a person skilled in the art without extending beyond the meaning and purpose of the claims.

In the following part some methods for obtaining the invention will be described, although equivalent materials and methods can be used. The following examples are therefore purely illustrative and non-limiting of the invention.

EXAMPLE 1

Cloning and characterisation of icIL-1raII

MATERIALS AND METHODS

Reagents

The following commercially available reagents were used for culture and separation of cells: pyrogenfree saline and distilled water for clinical use; RPMI 1640 medium; DMEM medium; M199 medium; L-glutamine; Percoll; Ficoll-Hipaque; aseptically collected fetal calf serum; endothelial cells growth supplement (ECGS), prepared from bovine brain; Heparin.

All reagents contained less than 0.125 EU/ml of endotoxin as checked by thr Limulus amebocyte lysate assay.

Cells

Human circulating PMN and monocytes were separated from the peripheral blood of healthy donors by centrifugation on a discontinuous (46% for monocytes and 62% for PMN) gradient of isoosmotic (285 mOsm) Percoll, as described in Colotta F., Peri G., Villa SA., Mantovani A., Rapid killing of actinomycin D treated tumour cells by human mononuclear cells. J. Immunol. 132:936, 1984. Cells were recovered at the interface, washed twice in saline and resuspended in the medium.

PMN and monocytes recovery was higher than 90% and purity higher than 98%, as assessed by morphological examination of stained cytocentrifuged cells. The cell culture medium routinely used for PMN and monocytes was RPMI 1640 with 2 mM L-glutamine and 10% FCS.

Human endothelial cells (EC) were obtained from umbilical veins and cultured, as described in detail in the literature (Allavena P., Paganin C., Martin-Padura I., Peri G., Gaboli M., Dejana E., Marchisio P.C., Mantovani A., Molecules and structures involved in the adhesion of natural killer cells to vascular endothelium, J. Exp. Med., 173:439, 1991).

Confluent cells at 2nd-5th passage maintained in M199 medium with 10% FCS supplemented with ECGS (50 µg/ml) and Heparin (100 µg/ml) were routinely used.

COS cells were cultivated in DMEM medium with 10% FCS and 8387 fibroblast cells in RPMI 1640 medium with 10% FCS.

After the appropriate treatment, cells were examined for IL-1ra mRNA or IL-1ra protein as described below.

RT-PCR

Total RNA was extracted by the guanidinium isothiocianate method with minor modifications.

RT-PCR was performed as described in Colotta F., Polentarutti N., Sironi M., Mantovani A., J. Biol. Chem., 267:18278, 1992.

Briefly, 1 μg total RNA was reverse transcribed in reverse transcriptase buffer (5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl; pH 8.3) with 2.5 mM random hexamers, 1 mM each deoxynucleotide triphosphate, 1 unit/ml RNase inhibitor, and 2.5 units/ml moloney murine leukaemia virus transcriptase (Perkin Elmer Cetus, Norwalk, CT).

Samples were incubated for 10 min at 25°C and then at 42°C for 45 min. Then, cDNA reaction was added with a specific pair of primers designed to amplify cDNAs encoding icIL-1raI or icIL-1raII and, as an internal control, human B-actin.

Amplification was carried out in 2 mM $MgCl_2$, 50 mM KCl, 0.2 M each deoxynucleotide triphosphate, 2.5 units/100 ml Taq polymerase (Perkin Elmer Cetus) and 4 mg/ml of each specific primer (see below). Amplification (30 cycles) was carried out in an automated thermal cycler (Perkin Elmer Cetus) at 95°C, at 55°C and at 72°C for 1.5 min each.

Amplified products were run through a 1% ethidium bromide-stained agarose gel along with molecular weight standards (Boehringer Mannheim, Mannheim, Germany).

Oligonucleotides were synthesised by the phosphoramidite method. The sequences of oligonucleotides used to selectively amplify icIL-1ra were identical to those described in Haskill S. et al., Natl. Acad., USA, 88:3681, 1991.

In particular, the authors used oligonucleotides GM397 (indicated here as IRA 1) and GM368 (IRA 4).

For icIL-1raII amplification the authors used IRA 4 and IRA 5 (SEQ ID NO:1), which specifically recognises the extra exon described here included in the icIL-1raII sequence.

For B-actin amplification the forward oligonucleotide is reported in SEQ ID NO:2, corresponding to nucleotides 60-79 of B-actin cDNA.

The backward oligonucleotide is reported in SEQ ID NO:3, complementary to nucleotides 430-449. Amplification products were subcloned (TA Cloning System, Invitrogen, San Diego, CA) and sequenced by the dideoxi chain termination method.

Expression of icIL-1ra products in COS cells

The cDNAs containing 32 bp of the 5'-untraslated region, the complete open reading frame and 6 bp (including the stop codon) of the 3'-untraslated region of both the icIL-1ral and icIL-1raII were obtained by RT-PCR with oligonucleotides IRA 4 and IRA 5 as detailed above and then ligated back into the pSF5 expression vector. Fidelity of reverse transcription and amplification was verified by sequencing.

The plasmids containing the cDNA in the correct orientation were purified on CsCl gradient and then transfected into COS cells by the calcium precipitate method as described in Sambrook J. et al., Cold Spring Harbor Laboratory Press, 1989.

After two days, culture supernatants and sonicated cell lysates examined by ELISA or immunoblotting as detailed below. An empty plasmid (not transfected) was used as a control.

Identification of immunoreactive IL-1ra

A commercial ELISA test (Amersham, Buckinamshire, UK) that identifies both sIL-1ra and icIL-1ra was used. For the Western blot analysis polyclonal antisera of two rabbits and of one goat were used.

COS cells lysates samples and supernatants were run on 12.5% SDS-PAGE electrophoresis and then blotted onto a nitro-cellulose filter (Stratagene, La Jolla, CA, USA).

Incubation with primary and secondary antibodies was carried out according to standard protocols. The primary antibody was an anti-IL-1ra rabbit polyclonal antibody.

The secondary antibody was a goat anti-rabbit immunoglobulin fraction linked to horseradish peroxidase (Amersham). Immunoreactive protein fraction bands were revealed by a chemiluminescence-based procedure (ECL Detection, Amersham) according to manufacturer's instructions.

Il-1-induced expression of E-selectin on EC

Confluent EC cultivated in 96 well plates (Falcon) were incubated for 30 minutes with an amount of transfected COS cells lysate (see above) corresponding to 25 to 100 ng of recombinant IL-1ra (either icIL-1ral or icIL-1raII) as assessed by a specific ELISA assay (Amersham).

As a control, an equal amount of COS lysate obtained from mock transfected cells was used in parallel. Next, EC were exposed for 6 hours to

0.1-1 ng/ml human recombinant IL-1B. The detection of E-selectin expression was made with an ELISA assay on adherent EC with the anti-E-selectin monoclonal antibody BB1G-E2 as primary antibody and a rabbit anti-mouse Ig antiserum conjugated with horseradish peroxidase as a secondary antibody. O.D. of the samples was determined by detecting the plates with a spectrophotometer (Flow) at 405 wavelength.

RESULTS

Identification of icIL-1raII

Specific oligonucleotide primers were designed (indicated as IRA 1 and IRA 4 in Fig.1) in order to obtain the whole coding sequence of icIL-1ra (Fig.1) by RT-PCR. Amplified products from human PMN were subcloned and sequenced.

In addition to the previously known sequence of icIL-1ra, the inventor isolated a number of clones whose sequences were identical to the published icIL-1ra coding sequence, with the notable exception of an extra sequence of 63 bp between nucleotides 132 and 133 of the icIL-1ra sequence. Given the described exon-intron boundaries of icIL-1ra, the extra sequence is inserted between the first leader-less exon of icIL-1ra and the internal acceptor site of the first exon of sIL-1ra (Fig.1).

The predicted amino acid sequence is shown in Fig.1. The new protein (thereafter referred to as icIL-1ra type II) has the first three amino acids at the NH_2 terminus in common with the classical icIL-1ra (icIL-1ra type I), followed by a new sequence of 21 amino acids. The rest of the two proteins is identical.

Curiously, the junction with the internal acceptor site of the first exon of sIL-1ra always generated, both for sIL-1ra and icIL-1ral and for icIL-1raII, the same amino acid residue, i.e. glutamic acid (Fig.1).

The most striking characteristic of the inserted amino acid extra sequence is the presence of seven glycin residues, six of which are consecutive. Glycin residues are flanked on both sides by glutamic acid residues. icIL-1raII consists of 180 amino acids.

The overall hydrophilic pattern of icIL-1raII is similar to that of icIL-1raI, still lacking an hydrophobic leader peptide at the NH_2 terminus.

Expression of icIL-raII

To identify icIL-1raII transcripts, RT-PCR analysis was performed with a pair of specifically designed oligonucleotides (IRA 5 and IRA 4, Fig.1), with an expected amplified product of 33 bp.

As shown in Fig.2, transcripts encoding icIL-1raII were detectable in PMA-, IL-1- and TNF-activated fibroblasts. A faint but detectable band was evident in LPS-treated monocytes.

Also PMN, either untreated or activated (Fig.2) showed a very faint band of the expected size.

The specificity of amplified products indicated in Fig.2 was confirmed by subcloning and sequencing.

Expression of recombinant icIL-1raII

COS cells were transfected with the DNA sequence encoding icIL-1raII and, by way of comparison, with that encoding icIL-1raI. Next, cell lysates and supernatants were examined by Western blot.

The polyclonal antisera used in these experiments recognised equally well icIL-1raII and icIL-1raI (Fig.3). Most, if not all, of icIL-1raII and icIL-1raI were found in cell lysates.

Recombinant icIL-1raI migrated as a predominant band of 22 KDa, whereas icIL-1raII showed a mass of approximately 25 KDa.

Inhibition of IL-1B activity by recombinant icIL-1raII

Recombinant icIL-1raII was examined for IL-1 inhibiting activity. To this aim the authors chose the IL-1-induced expression of E-selectin on endothelial cells, because this assay is sensitive (detectable induction at 100 pg/ml IL-1, or less) and rapid (6 hours incubation with IL-1).

Lysates of mock transfected COS cells did not significatively reduce the IL-1 activity.

icIL-1raII had no agonistic activity.

As shown in Fig.4, recombinant icIL-1raII inhibited in a dose-dependent fashion IL-1 activity.

These data provide evidence that icIL-1raII is indeed an inhibitor of IL-1.

DISCUSSION

The inventors describe a new molecular form of icIL-1ra. The new molecule is generated by insertion of 63 bp between the first leader-less exon of icIL-1ra and the internal acceptor site of the first exon of sIL-1ra.

Since the resulting protein is partially identical to classical icIL-1ra, with the exception of an extra sequence of 21 amino acids located in the NH_2 terminus of the molucule, the inventors suggest to term this new form as IL-1ra type II, referring to the classical icIL-1ra sequence as icIL-1ra type I.

RT-PCR experiments demonstrated that icIL-1raII transcripts are inducible in monocytes and fibroblasts. Recombinant icIL-1raII expressed in COS cells had an apparent MW of approximately 25 KDa and an inhibitor activity of IL-1 comparable to that exerted by icIL-1raI expressed under the same experimental conditions.

Transcripts coding for icIL-1ra and sIL-1ra are generated from the same gene by means of usage of differential splicing. icIL-1ra is generated by an alternative start of transcription of an exon inserted into an internal acceptor site of the first exon containing the leader sequence of sIL-1ra.

The results obtained by the inventors suggest a new organisation of IL-1ra gene, in which an extra exon is located between the first exon of, respectively, classical icIL-1ra and sIL-1ra. Use of this new exon generates a polypeptide molecule which, still lacking a signal peptide, differs from icIL-1raI at its N terminus by the insertion of 21 amino acids, still remaining inhibitory capacity against IL-1.

Use of alternative splicing to generate different IL-1ra molecules appears to be highly regulated. icIL-1raII transcripts were induced by IL-1, TNF and phorbol esters in fibroblasts and by LPS in monocytes. In fibroblasts, phorbol esters were found to selectively induce icIL-1ra transcripts, whereas IL-1 and TNF induced both sIL-1ra and icIL-1ra mRNAs. In monocytes, IL-13, which augmented both transcripts of sIL-1ra and icIL-1raI, failed to induce icIL-1raII.

Finally, PMN, in which sIL-1ra and icIL-1ra are costitutively expressed and inducible, expressed very few transcripts, as pointed out by RT-PCR. Overall, these data indicate that the mechanisms inducing the differential splicing generating the three forms of IL-1ra are differentially regulated in response to external signals.

The amino acid sequence of the extra sequence described here is surprising in that it contains seven residues of glycin, six of which are consecutive.

Glycin-rich sequences are present in molecules with different biological activities, including the atrial natriuretic clearance receptor, the HOX11 home box gene, the intermediate filaments keratins and nuclear proteins involved in centromere binding or RNA splicing.

Apart from glycin residues, however, no obvious homology was evident between these proteins and icIL-1raII in the amino acid sequence flanking glycin-rich regions.

IL-1 system shows an extraordinary level of complexity, consisting of two agonists, two receptors, one of which is an inhibitor of IL-1, and a receptor antagonist, for which at least three different molecular forms could exist taking into account the results obtained.

Although the biological significance of the intracellular forms of IL-1ra remains to be clearly established, the data here reported indicate that by alternative splicing two different forms of icIL-1ra can be generated in response to selected external stimuli, with different N termini.

The existence of multiple and complex levels of control of IL-1 points to the absolute requirement for a tight physiological control of the inflammatory potential of this cytokine.

DESCRIPTION OF FIGURES

Figure 1

DNA sequence and predicted protein sequence of icIL-1raII compared to classical icIL-1ra(icIL-1raI) and sIL-1ra.

The upper part of Figure 1 shows DNA and protein sequences specifically represented in sIL-1ra, icIL-1raI and icIL-1raII. The lower part of Figure 1 shows the sequence in common among the three forms of IL-1ra.

The entire sequences for each molecule are thus generated by the junction of each specific portion with the common sequence. For clarity, the DNA sequence of icIL-1ra starts from nucleotide 91 of the published 5' untraslated sequence, and only 6 bp of the 3' untraslated sequence are reported.

The common IL-1ra sequence starts with the internal acceptor site located in the first exon of sIL-1ra, corresponding to nucleotide 133 of the complete icIL-1raI sequence and to nucleotide 88 of the complete sIL-1ra sequence.

Arrows indicate forward (IRA 1 and IRA 5) and backward (IRA 4) oligonucleotides used for RT-PCR analysis, as described in the text. The oligonucleotide IRA 5 recognises only icIL-1raII DNA.

Figure 2

RT-PCR analysis of icIL-1raII expression in different cell types

RNAs from 8387 fibroblasts (panel A), monocytes (B) and PMN (C) were reverse-transcribed. Each DNA synthesis reaction was then divided in two samples, one of which amplified with oligonucleotides IRA 5 (forward) and IRA 4 (backward) for detection of icIL-1raII transcripts, and the other amplified with B-actin specific oligonucleotides (see Material and Methods Section).

Amplified products were then examined through an ethidium bromide-stained agarose gel. Amplified products corresponding to B-actin are reported on the left side of the standard and the amplified products corresponding to icIL-1raII (on the right) are indicated by a arrow. The specificity of these bands was confirmed by subcloning and sequencing.

Figure 3

Western blot analysis of recombinant icIL-1raII

Cell lysates from COS cells transfected with DNAs encoding icIL-1raI (2) or icIL-1raII (3) or with an empty vector which does not contain such DNA (1) were examined by immunoblotting with an anti-IL-1ra rabbit polyclonal antibody. Molecular weight standards are indicated.

Figure 4

Effects of icIL-1raII on IL-1-induced expression of E-selectin on endothelial cells

Endothelial cells were treated with 0.1 or 1 ng/ml of human IL-1B, with or without 25-100 ng/ml of icIL-1raII or equivalent amounts of COS cell lysates obtained from cells which were mock transfected by means of an empty vector, as explained in details in the Material and Method section.

After 6 hours of incubation, the endothelial cells were examined for E-selectin expression by an ELISA test performed on adherent cells.

The data reported are percentages of IL-1-induced E-selectin expression for the control.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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(ii) TITLE OF INVENTION: INTERLEUKIN-1 ANTAGONIST

(iii) NUMBER OF SEQUENCES: 14

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(B) LOCATION:1..25

(D)OTHER INFORMATION:/note= "RT-PCR oligonucleotide named IRA5"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

CTGACTTGTA TGAAGAAGGA GGTGG 25

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(B) LOCATION:1..20

(D) OTHER INFORMATION:/note= "RT-PCR oligonucleotide corresponding to 60-79 of B-actin"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

GCGCTCGTCG TCGACAACGG 20

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(B) LOCATION:1..21

(D) OTHER INFORMATION:/note= "RT-PCR backward oligonucleotide complementary to 430-449"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GATAGACAAC GTACATGGCT G 21

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 87 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION:24..86

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(B) LOCATION:1..87

(D) OTHER INFORMATION:/note= "Sequence of sIL-1ra not in common"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

```
GAATTCCGGG CTGCAGTCAC AGA ATG GAA ATC TGC AGA GGC CTC CGC AGT        50
                          Met Glu Ile Cys Arg Gly Leu Arg Ser
                            1               5

CAC CTA ATC ACT CTC CTC CTC TTC CTG TTC CAT TCA G                    87
His Leu Ile Thr Leu Leu Leu Phe Leu Phe His Ser
10                  15                  20
```

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

```
Met Glu Ile Cys Arg Gly Leu Arg Ser His Leu Ile Thr Leu Leu Leu
 1               5                  10                  15

Phe Leu Phe His Ser
            20
```

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 42 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION:33..41

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(B) LOCATION:1..42

(D) OTHER INFORMATION:/note= "Sequence of intracellular IL-1ra type I not in common"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

CAGAAGACCT CCTGTCCTAT GAGGCCCTCC CC ATG GCT TTA G 42

Met Ala Leu

1

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 3 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Met Ala Leu

1

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 105 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION:33..104

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(B) LOCATION:1..105

(D) OTHER INFORMATION:/note= "Sequence of intracellular IL-1ra type II not in common"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

```
CAGAAGACCT CCTGTCCTAT GAGGCCCTCC CC ATG GCT TTA GCT GAC TTG TAT     53
                                    Met Ala Leu Ala Asp Leu Tyr
                                      1               5

GAA GAA GGA GGT GGA GGA GGA GGA GAA GGT GAA GAC AAT GCT GAC TCA     101
Glu Glu Gly Gly Gly Gly Gly Gly Glu Gly Glu Asp Asn Ala Asp Ser
         10                  15                  20

AAG G                                                              105
Lys
```

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

```
Met Ala Leu Ala Asp Leu Tyr Glu Glu Gly Gly Gly Gly Gly Gly Glu
  1               5                  10                  15

Gly Glu Asp Asn Ala Asp Ser Lys
            20
```

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 474 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION:1..468

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(B) LOCATION:1..474

(D) OTHER INFORMATION:/note= "Common IL-1ra seq.; a G was added in the first position for software reason, so as the first codon codes for Glu and so as the creation of a stop codon in the inner region of the seq. is avoided"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

```
GAG ACG ATC TGC CGA CCC TCT GGG AGA AAA TCC AGC AAG ATG CAA GCC    48
Glu Thr Ile Cys Arg Pro Ser Gly Arg Lys Ser Ser Lys Met Gln Ala
 1               5                  10                  15

TTC AGA ATC TGG GAT GTT AAC CAG AAG ACC TTC TAT CTG AGG AAC AAC    96
Phe Arg Ile Trp Asp Val Asn Gln Lys Thr Phe Tyr Leu Arg Asn Asn
            20                  25                  30

CAA CTA GTT GCT GGA TAC TTG CAA GGA CCA AAT GTC AAT TTA GAA GAA   144
Gln Leu Val Ala Gly Tyr Leu Gln Gly Pro Asn Val Asn Leu Glu Glu
        35                  40                  45

AAG ATA GAT GTG GTA CCC ATT GAG CCT CAT GCT CTG TTC TTG GGA ATC   192
Lys Ile Asp Val Val Pro Ile Glu Pro His Ala Leu Phe Leu Gly Ile
    50                  55                  60

CAT GGA GGG AAG ATG TGC CTG TCC TGT GTC AAG TCT GGT GAT GAG ACC   240
His Gly Gly Lys Met Cys Leu Ser Cys Val Lys Ser Gly Asp Glu Thr
65                  70                  75                  80

AGA CTC CAG CTG GAG GCA GTT AAC ATC ACT GAC CTG AGC GAG AAC AGA   288
Arg Leu Gln Leu Glu Ala Val Asn Ile Thr Asp Leu Ser Glu Asn Arg
```

```
                85                  90                  95
AAG CAG GAC AAG CGC TTC GCC TTC ATC CGC TCA GAC AGT GGC CCC ACC   336
Lys Gln Asp Lys Arg Phe Ala Phe Ile Arg Ser Asp Ser Gly Pro Thr
            100                 105                 110
ACC AGT TTT GAG TCT GCC GCC TGC CCC GGT TGG TTC CTC TGC ACA GCG   384
Thr Ser Phe Glu Ser Ala Ala Cys Pro Gly Trp Phe Leu Cys Thr Ala
        115                 120                 125
ATG GAA GCT GAC CAG CCC GTC AGC CTC ACC AAT ATG CCT GAC GAA GGC   432
Met Glu Ala Asp Gln Pro Val Ser Leu Thr Asn Met Pro Asp Glu Gly
    130                 135                 140
GTC ATG GTC ACC AAA TTC TAC TTC CAG GAG GAC GAG TAGTAC       474
Val Met Val Thr Lys Phe Tyr Phe Gln Glu Asp Glu
145
```

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 156 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

```
Glu Thr Ile Cys Arg Pro Ser Gly Arg Lys Ser Ser Lys Met Gln Ala
  1               5                  10                  15
Phe Arg Ile Trp Asp Val Asn Gln Lys Thr Phe Tyr Leu Arg Asn Asn
             20                  25                  30
Gln Leu Val Ala Gly Tyr Leu Gln Gly Pro Asn Val Asn Leu Glu Glu
         35                  40                  45
Lys Ile Asp Val Val Pro Ile Glu Pro His Ala Leu Phe Leu Gly Ile
     50                  55                  60
His Gly Gly Lys Met Cys Leu Ser Cys Val Lys Ser Gly Asp Glu Thr
65                  70                  75                  80
Arg Leu Gln Leu Glu Ala Val Asn Ile Thr Asp Leu Ser Glu Asn Arg
                85                  90                  95
Lys Gln Asp Lys Arg Phe Ala Phe Ile Arg Ser Asp Ser Gly Pro Thr
            100                 105                 110
```

```
Thr Ser Phe Glu Ser Ala Ala Cys Pro Gly Trp Phe Leu Cys Thr Ala
        115                 120                 125
Met Glu Ala Asp Gln Pro Val Ser Leu Thr Asn Met Pro Asp Glu Gly
    130                 135                 140
Val Met Val Thr Lys Phe Tyr Phe Gln Glu Asp Glu
145                 150                 155
```

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:

(A) NAME/KEY: Peptide

(B) LOCATION:1..21

(D) OTHER INFORMATION:/note= "A portion of the intracellular IL-1ra type II not in common"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

```
Ala Asp Leu Tyr Glu Glu Gly Gly Gly Gly Gly Gly Gly Gly Glu Asp
  1               5                  10                  15
Asn Ala Asp Ser Lys
            20
```

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 579 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION:34..573

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(B) LOCATION:1..579

(D) OTHER INFORMATION:/note= "Intracellular IL-1ra type II"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

```
CAGAAGGACC TCCTGTCCTA TGAGGCCCTC CCC ATG GCT TTA GCT GAC TTG TAT    54
                                     Met Ala Leu Ala Asp Leu Tyr
                                       1               5

GAA GAA GGA GGT GGA GGA GGA GGA GAA GGT GAA GAC AAT GCT GAC TCA    102
Glu Glu Gly Gly Gly Gly Gly Gly Glu Gly Glu Asp Asn Ala Asp Ser
         10                  15                  20

AAG GAG ACG ATC TGC CGA CCC TCT GGG AGA AAA TCC AGC AAG ATG CAA    150
Lys Glu Thr Ile Cys Arg Pro Ser Gly Arg Lys Ser Ser Lys Met Gln
     25                  30                  35

GCC TTC AGA ATC TGG GAT GTT AAC CAG AAG ACC TTC TAT CTG AGG AAC    198
Ala Phe Arg Ile Trp Asp Val Asn Gln Lys Thr Phe Tyr Leu Arg Asn
 40                  45                  50                  55

AAC CAA CTA GTT GCT GGA TAC TTG CAA GGA CCA AAT GTC AAT TTA GAA    246
Asn Gln Leu Val Ala Gly Tyr Leu Gln Gly Pro Asn Val Asn Leu Glu
                 60                  65                  70

GAA AAG ATA GAT GTG GTA CCC ATT GAG CCT CAT GCT CTG TTC TTG GGA    294
Glu Lys Ile Asp Val Val Pro Ile Glu Pro His Ala Leu Phe Leu Gly
             75                  80                  85

ATC CAT GGA GGG AAG ATG TGC CTG TCC TGT GTC AAG TCT GGT GAT GAG    342
Ile His Gly Gly Lys Met Cys Leu Ser Cys Val Lys Ser Gly Asp Glu
         90                  95                  100

ACC AGA CTC CAG CTG GAG GCA GTT AAC ATC ACT GAC CTG AGC GAG AAC    390
Thr Arg Leu Gln Leu Glu Ala Val Asn Ile Thr Asp Leu Ser Glu Asn
     105                 110                 115
```

```
AGA AAG CAG GAC AAG CGC TTC GCC TTC ATC CGC TCA GAC AGT GGC CCC     438
Arg Lys Gln Asp Lys Arg Phe Ala Phe Ile Arg Ser Asp Ser Gly Pro
120                 125                 130                 135
ACC ACC AGT TTT GAG TCT GCC GCC TGC CCC GGT TGG TTC CTC TGC ACA     486
Thr Thr Ser Phe Glu Ser Ala Ala Cys Pro Gly Trp Phe Leu Cys Thr
                140                 145                 150
GCG ATG GAA GCT GAC CAG CCC GTC AGC CTC ACC AAT ATG CCT GAC GAA     534
Ala Met Glu Ala Asp Gln Pro Val Ser Leu Thr Asn Met Pro Asp Glu
            155                 160                 165
GGC GTC ATG GTC ACC AAA TTC TAC TTC CAG GAG GAC GAG TAGTAC          579
Gly Val Met Val Thr Lys Phe Tyr Phe Gln Glu Asp Glu
        170                 175                 180
```

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 180 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

```
Met Ala Leu Ala Asp Leu Tyr Glu Glu Gly Gly Gly Gly Gly Gly Glu
  1               5                  10                  15
Gly Glu Asp Asn Ala Asp Ser Lys Glu Thr Ile Cys Arg Pro Ser Gly
             20                  25                  30
Arg Lys Ser Ser Lys Met Gln Ala Phe Arg Ile Trp Asp Val Asn Gln
         35                  40                  45
Lys Thr Phe Tyr Leu Arg Asn Asn Gln Leu Val Ala Gly Tyr Leu Gln
     50                  55                  60
Gly Pro Asn Val Asn Leu Glu Glu Lys Ile Asp Val Val Pro Ile Glu
65                  70                  75                  80
Pro His Ala Leu Phe Leu Gly Ile His Gly Gly Lys Met Cys Leu Ser
                85                  90                  95
Cys Val Lys Ser Gly Asp Glu Thr Arg Leu Gln Leu Glu Ala Val Asn
            100                 105                 110
Ile Thr Asp Leu Ser Glu Asn Arg Lys Gln Asp Lys Arg Phe Ala Phe
```

115 120 125

Ile Arg Ser Asp Ser Gly Pro Thr Thr Ser Phe Glu Ser Ala Ala Cys

130 135 140

Pro Gly Trp Phe Leu Cys Thr Ala Met Glu Ala Asp Gln Pro Val Ser

145 150 155 160

Leu Thr Asn Met Pro Asp Glu Gly Val Met Val Thr Lys Phe Tyr Phe

165 170 175

Gln Glu Asp Glu

180

CLAIMS

1. Purified protein having antagonist activity against at least one of the substances selected from the group consisting of interleukin a and interleukin B, or fragments thereof, characterised by the fact of comprising the amino acid sequence reported in SEQ ID NO:12.

2. A purified protein having antagonist activity against at least one of the substances selected from the group consisting of interleukin a and interleukin B, or fragments thereof, characterised by the fact to have the amino acid sequence reported in SEQ ID NO:14.

3. A purified protein according to claim 2, characterised by the fact of being obtained by recombinant DNA technique.

4. Isolated DNA sequence encoding a IL-1 antagonist having the amino acid sequence according to claim 2.

5. Isolated DNA sequence according to claim 4, characterised by the fact of having reported in SEQ ID NO:13.

6. Isolated DNA sequence that ibridizes with the probe having the sequence reported in SEQ ID NO:1. .

7. Vector comprising the DNA sequence encoding the protein according to claim 2.

8. Cell culture transfected with DNA encoding the protein according to claim 2.

9. Cell culture according to claim 2, characterised by the fact of being obtained by mammalian cells.

10. Process for obtaining a IL-1 antagonist by the recombinant DNA technique comprising:
a) cultivating host cells as described in claims 8 and 9 containing a DNA sequence able to produce the protein according to claim 2;

b) collecting and isolating the encoded protein.

11. Process of obtaining a IL-1 antagonist by recombinant DNA technique, characterised by the fact that the DNA sequence is that reported in SEQ ID NO:13.

12. Use of a purified protein having the protein according to claim 2, for medical use.

13. Use of a purified protein having the sequence according to claim 2 for the preparation of pharmaceutical compositions active in pathologies requiring IL-1 inhibition.

14. Use according to claim 13, characterised by the fact that the pathology is selected from the group of autoimmune pathologies.

15. Use according to claim 14, characterised by the fact that the pathology is selected from the group consisting of rheumatoid arthritis, septic shock, acute myelomonocytic leukaemia, immunological reaction of transplantation against host, acquired immunodeficiency syndrome (AIDS), ulcerative colitis.

Figure 1

Secreted IL-1ra

```
GAATTCCGGG CTGCAGTCAC AGA ATG GAA ATC TGC AGA GGC CTC CGC AGT CAC CTA ATC ACT CTC CTC CTC TTC CTG TTC CAT TCA G
                          Met Glu Ile Cys Arg Gly Leu Arg Ser His Leu Ile Thr Leu Leu Leu Phe Leu Phe His Ser
```

Intracellular IL-1ra type I

```
          IRA 1
|------------------------>
CAGAAGACCT CCTGTCCTAT GAGGCCCTCC CC ATG GCT TTA G
                                   Met Ala Leu
```

Intracellular IL-1ra type II

```
          IRA 1                                          IRA 5
|------------------------>                  |------------------------------->
CAGAAGACCT CCTGTCCTAT GAGGCCCTCC CC ATG GCT TTA GCT GAC TTG TAT GAA GAA GGA GGT GGA GGA GGA GGA GAA GGT
                                   Met Ala Leu Ala Asp Leu Tyr Glu Glu Gly Gly Gly Gly Gly Gly Glu Gly

GAA GAC AAT GCT GAC TCA AAG G
Glu Asp Asn Ala Asp Ser Lys
```

Common IL-1ra sequence

```
 AG ACG ATC TGC CGA CCC TCT GGG AGA AAA TCC AGC AAG ATG CAA GCC TTC AGA ATC TGG GAT GTT AAC CAG AAG ACC
Glu Thr Ile Cys Arg Pro Ser Gly Arg Lys Ser Ser Lys Met Gln Ala Phe Arg Ile Trp Asp Val Asn Gln Lys Thr

TTC TAT CTG AGG AAC AAC CAA CTA GTT GCT GGA TAC TTG CAA GGA CCA AAT GTC AAT TTA GAA GAA AAG ATA GAT GTG
Phe Tyr Leu Arg Asn Asn Gln Leu Val Ala Gly Tyr Leu Gln Gly Pro Asn Val Asn Leu Glu Glu Lys Ile Asp Val

GTA CCC ATT GAG CCT CAT GCT CTG TTC TTG GGA ATC CAT GGA GGG AAG ATG TGC CTG TCC TGT GTC AAG TCT GGT GAT
Val Pro Ile Glu Pro His Ala Leu Phe Leu Gly Ile His Gly Gly Lys Met Cys Leu Ser Cys Val Lys Ser Gly Asp
(continues)
```


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Figure 1 cont.

```
GAG ACC AGA CTC CAG CTG GAG GCA GTT AAC ATC ACT GAC CTG AGC GAG AAC AGA AAG CAG GAC AAG CGC TTC GCC TTC
Glu Thr Arg Leu Gln Leu Glu Ala Val Asn Ile Thr Asp Leu Ser Glu Asn Arg Lys Gln Asp Lys Arg Phe Ala Phe

ATC CGC TCA GAC AGT GGC CCC ACC ACC AGT TTT GAG TCT GCC GCC TGC CCC GGT TGG TTC CTC TGC ACA GCG ATG GAA
Ile Arg Ser Asp Ser Gly Pro Thr Thr Ser Phe Glu Ser Ala Ala Cys Pro Gly Trp Phe Leu Cys Thr Ala Met Glu

GCT GAC CAG CCC GTC AGC CTC ACC AAT ATG CCT GAC GAA GGC GTC ATG GTC ACC AAA TTC TAC TTC CAG GAG GAC GAG
Ala Asp Gln Pro Val Ser Leu Thr Asn Met Pro Asp Glu Gly Val Met Val Thr Lys Phe Tyr Phe Gln Glu Asp Glu
                                                                                <----------------
                                                                                IRA 4

TAGTAC

------I
```

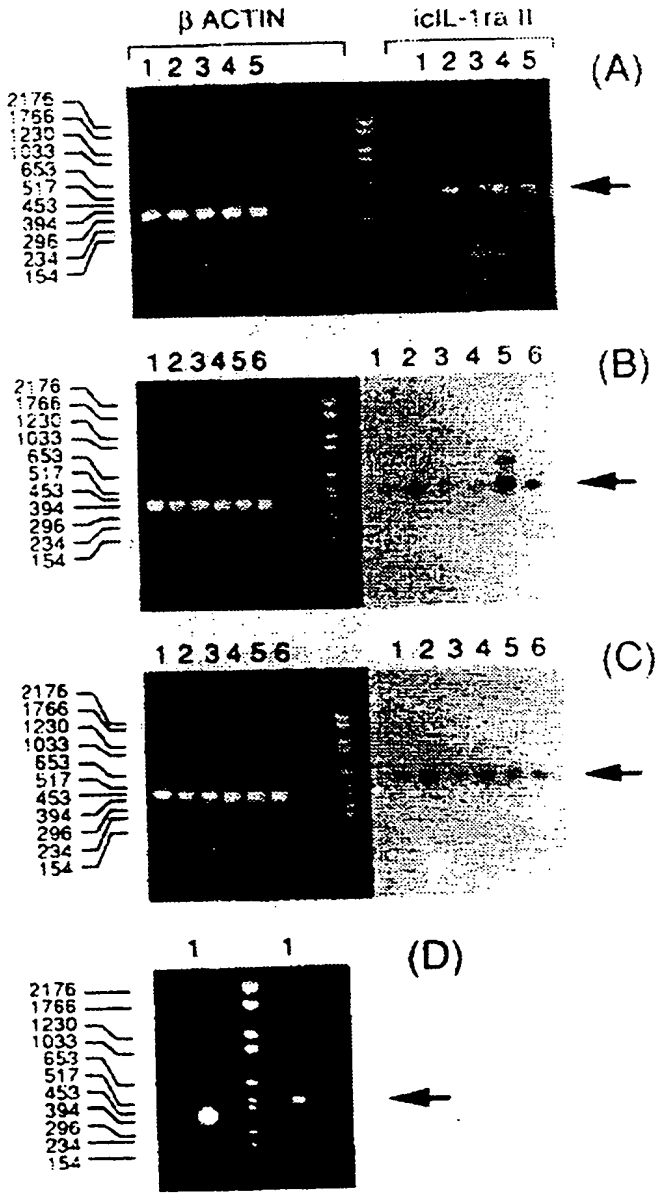
β ACTIN
icIL-1ra II
1 2 3 4 5
1 2 3 4 5
(A)
2176
1766
1230
1033
653
517
453
394
296
234
154
1 2 3 4 5 6
1 2 3 4 5 6
(B)
1 2 3 4 5 6
1 2 3 4 5 6
(C)
1
1
(D)

Figure 2

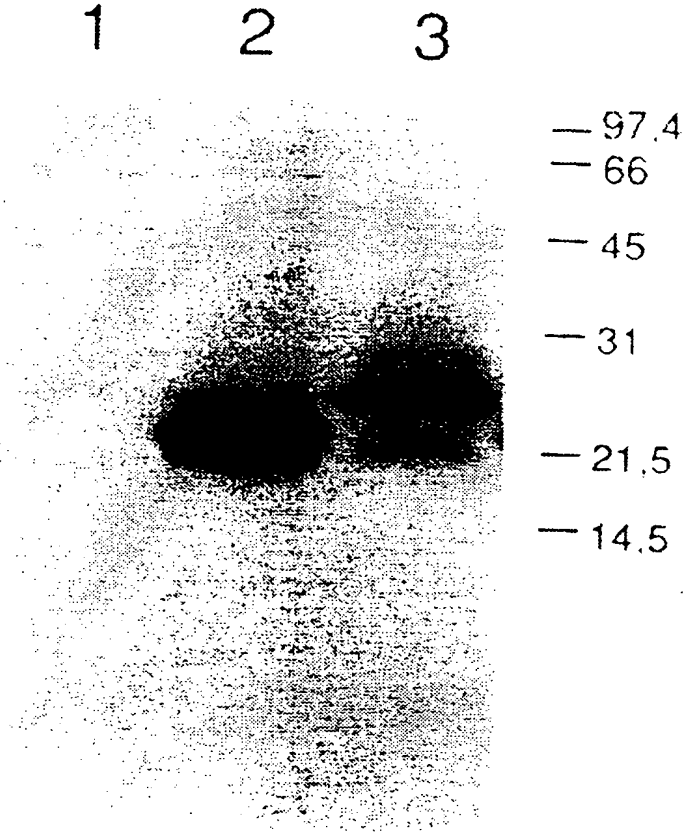

Figure 3

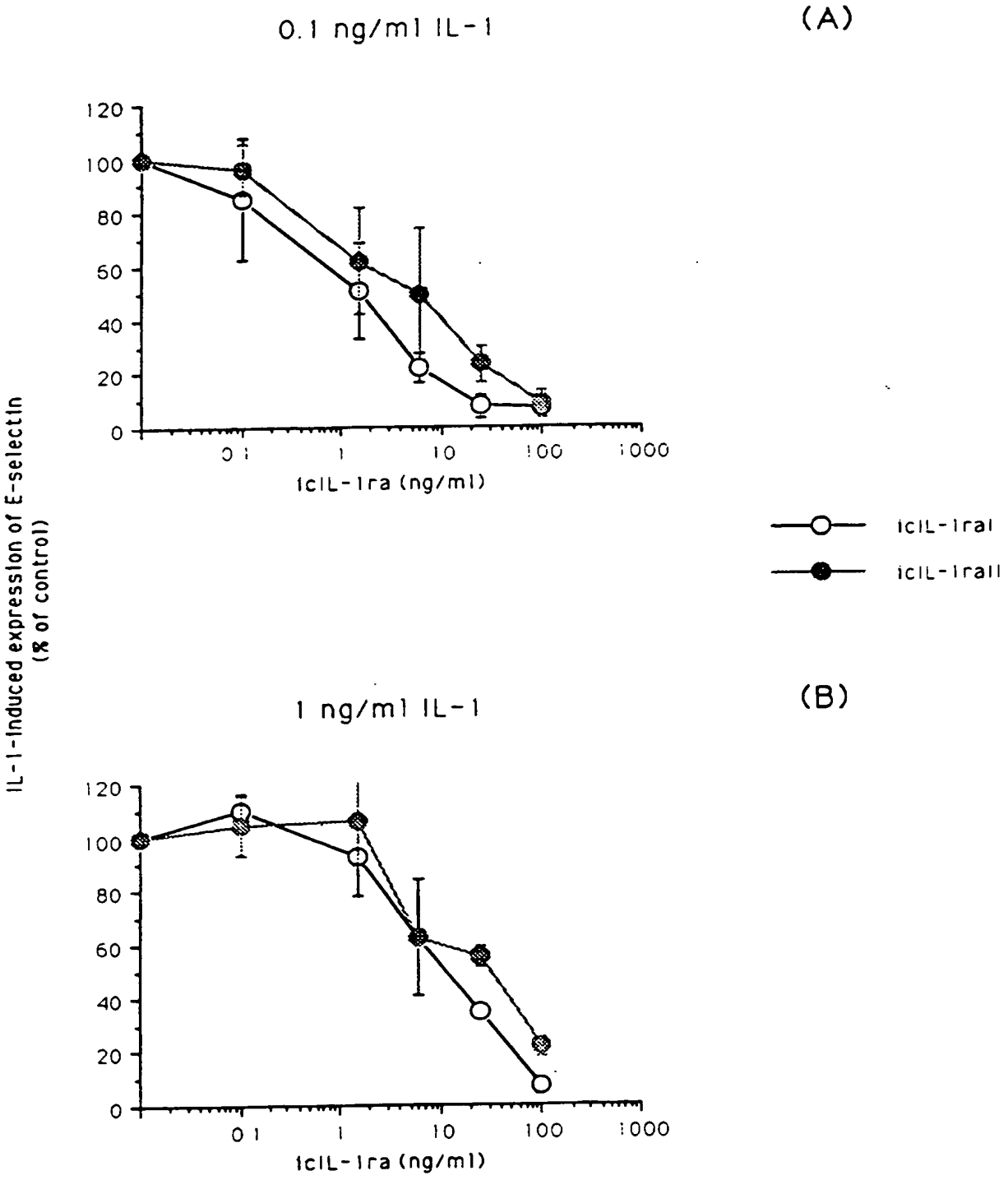
0.1 ng/ml IL-1
(A)
120
100
80
60
40
20
0
0.1
1
10
100
1000
icIL-1ra (ng/ml)
IL-1-induced expression of E-selectin
(% of control)
icIL-1raI
icIL-1raII
1 ng/ml IL-1
(B)
120
100
80
60
40
20
0
0.1
1
10
100
1000
icIL-1ra (ng/ml)

Figure 4

INTERNATIONAL SEARCH REPORT

[redacted]mation[redacted]ication No
PCT/EP 95/04023

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/24 C07K14/54 C12N5/10 A61K38/20

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 88, 1991 WASHINGTON US, pages 3681-3685, S. HASKILL ET AL 'cDNA cloning of an intracellular form of the human Interleukin 1 receptor antagonist associated with epithelium' cited in the application see the whole document --- -/--	1-11

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 February 1996	15.03.96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Le Cornec, N

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JOURNAL OF IMMUNOLOGY, vol. 153, July 1994 BALTIMORE US, pages 701-711, C. BUTCHER ET AL 'Comparison of two promoters controlling expression of secreted or intracellular il-1 receptor antagonist' see the whole document	1-12
A	IMMUNOLOGY TODAY, vol. 12, no. 11, 1991 CAMBRIDGE GB, pages 404-410, C. A. DINARELLO ET AL 'Blocking il-1 : Interleukin 1 receptor antagonist in vivo and in vitro' see the whole document	1,2, 12-15
X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 85, May 1988 WASHINGTON US, pages 2929-2933, N. F. ZANDER ET AL 'cDNA cloning and complete primary structure of skeletal muscle phosphorylase kinase(alpha subunit)' see figure 2	6
X	EMBL database entry SKADECYC Accession number X56042 (version 1);19 november 1990; YOUNG D. et al see abstract	6
A	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 88, June 1991 WASHINGTON US, pages 5232-5236, S.P. EISENBERG ET AL 'INterleukin 1 receptor antagonist is a member of the interleukin 1 gene family : Evolution of a cytokine control mechanism'	
A	WO,A,91 17249 (CETUS CORPORATION) 14 November 1991 see abstract; claims; table I see page 29; figure 2	1-15
P,X	JOURNAL OF EXPERIMENTAL MEDICINE, vol. 182, no. 2, 1 August 1995 pages 623-628, M. MUZIO ET AL 'Cloning and characterization of a new isoform of the interleukin 1 receptor antagonist' see the whole document	1-12

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 95/04023

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 12
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim 12 is directed to a method of treatment of the human/animal body (rule 39.1(iv)PCT) the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

:rnational Application No
PCT/EP 95/04023

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9117249	14-11-91	AU-B-	655766	12-01-95
		AU-B-	7692791	27-11-91
		CA-A-	2081774	02-11-91
		EP-A-	0534978	07-04-93
		US-A-	5455330	03-10-95